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(54) Title: ENHANCED OIL RECOVERY

(57) Abstract

The present invention relates to the use of lactic acid bacteria in oil recovery operations. The invention provides a method of stimulating oil recovery from an oil reservoir which comprises: (i) injecting an inoculum of a lactic acid bacterium compatible with the reservoir conditions into a well bore drilled into the reservoir; (ii) injecting a source of nutrients for the bacterium into the reservoir; (iii) allowing the bacterium to ferment thereby producing lactic acid; and (iv) recovering oil from the reservoir. Particularly preferred bacteria are those of the genera *Lactobacillus* or *Pediococcus*. Lactic acid produced by bacteria may also be used for removal of carbonate or iron scale in oilfield equipment.

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ENHANCED OIL RECOVERY

The present invention relates to the use of bacteria in the production of oil from an oil well.

One third to a half of the world's oil reserves are found in carbonate rock structures, in particular a large area of the Texas oilfields in the US, the oil reservoirs of Oman and many onshore areas in Europe. Carbonate rock tends to have very low permeability particularly when there are no fractures present.

In many sandstone reservoirs, the rock structure may be cemented together by carbonate, or carbonate scales may accumulate close to production wells as a result of carbon dioxide being released from solution due to the presence of a pressure drop. Another type of scale that can accumulate around production wells is iron scale, in particular iron oxides and hydroxides. Low permeability, drilling damage and accumulation of scale all impede the flow of oil to the production well and the conventional method used to open up channels around the well bore to improve the flow rate is the injection of acid (known as acidising or acid stimulation). There are two types of acid treatment: fracture acidising (injection of acid at rates above the fracture pressure to etch the faces of the resultant fractures) and matrix acidising (injection of acid at rates below the fracture pressure to dissolve flow channels in the rock or to remove scale or damage caused by drilling). Acid treatments are employed in all types of oil wells: they may be used to open fractures or remove damage in newly drilled wells or to rehabilitate old wells from

which production has declined. Acid (usually hydrochloric acid though acetic and formic acids are used on occasion) is pumped into the well, where it reacts with the calcium carbonate according to the following stoichiometry:

$$CaCO_3 + 2HCl \longrightarrow CaCl_2 + CO_2 + H_2O$$

Calcium chloride ($CaCl_2$) is highly soluble in water and the acid attack etches channels in the rock which improves the oil flow towards the production well. Conventionally oil wells in carbonate reservoirs are acidised immediately after drilling before production commences and often repeat treatments are conducted every two to three years.

The conventional acidisation technique has the advantage that it is quick, usually taking less than a day, and the raw material is cheap. However it has the disadvantages that the hydrochloric acid is highly corrosive involving expensive and careful handling when being transported to the well and pumped downhole. Hydrochloric acid also corrodes the downhole equipment, such as pumps, casing and tubulars made of steel, chrome or aluminium. It requires the addition of sequestering agents to prevent iron precipitation in the reservoir and corrosion inhibitors to limit the damage to equipment. These chemicals increase the complexity and the price of the treatment. A further disadvantage is that the acid reacts too quickly in carbonate reservoirs and is frequently spent very close to the well bore (in the first few feet) and consequently does not open channels deeper into the formation.

The use of bacteria to improve oil recovery in a wide variety of oil fields has been proposed. US-A-4,475,590 outlines a number of ways in which microorganisms are believed to enhance oil recovery. These include: (a) reducing viscosity by degrading higher molecular weight hydrocarbons; (b) producing organic acids which dissolve cementing materials in the formation; (c) producing surfactants; (d) physically displacing the oil adhering to particles of sand in the formation; or (e) plugging the most porous portions of a reservoir, thereby reducing the tendency of water to "finger" through the reservoir.

These proposed uses have been in connection with secondary and tertiary oil recovery. Despite the known problems associated with hydrochloric acid, this acid is frequently the only treatment available for wells with a declining primary production.

Attempts to use organic acid producing bacteria in oil recovery operations have to date focused on the use of species which produce acids such as acetic acid. For example, DD-A-322000 discloses the use of Clostridium and Bacillus species, which primarily produce acetic acid, as a useful oil recovery agents in carbonate deposits. Grula, E.A. et al (Microbes and Oil Recovery (Vol. 1), International Bioresources Journal, Ed Zajic and Donaldson, 1985) also indicate that Clostridium is useful for enhanced oil recovery.

According to the present invention, there is provided a method of stimulating oil recovery from an oil reservoir which comprises:

(i) injecting an inoculum of a lactic acid

bacterium compatible with the reservoir conditions into a well bore drilled into the reservoir;

(ii) injecting a source of nutrients for the bacterium into the reservoir;

(iii) allowing the bacterium to ferment thereby producing lactic acid; and

(iv) recovering oil from the reservoir.

The inoculum and/or nutrients may be injected at either above, at, or below the reservoir fracture pressure. The pressure may be varied during injection.

In general, the bacteria for use in the invention will be those in which more than 50%, and usually more than 75%, of the acid produced during fermentation is lactic acid. Indeed, lactic acid producing bacteria are capable of converting very high proportions (as much as 99%) of carbon substrate into acid.

Lactic acid is produced by the lactic acid bacteria, which include the bacterial genera Lactobacillus, Pediococcus, Streptococcus and Leuconostoc, as a product of fermentative metabolism. The lactic acid bacteria are facultative anaerobes with a high tolerance to acid, growth occurs at pH values below 5.0. These lactic acid bacteria do not form spores. Lactic acid is the major acid produced during fermentation and for many species (homofermenters) the only product. Homofermenters are capable of converting glucose quantitively to lactic acid, heterofermenters to an equimolar mixture of lactic acid, ethanol and carbon dioxide. Certain species of spore forming bacteria including Sporolactobacillus inulinis and Bacillus (such as Bacillus laevolacticus) are

capable of homofermentative conversion of glucose to lactic acid. These organisms are also considered to be lactic acid bacteria for the purposes of this invention. The fermentation involves the anaerobic (oxygen independent) breakdown of carbon substrate such as sugars to lactic acid as a means of providing energy for growth.

We have found that the lactic acid bacteria according to the invention can act as an in situ substitute for hydrochloric acid treatment. The stoichiometry of the reaction between calcium carbonate and lactic acid is as follows:

$$CaCO_3 + 2CH_3CH(OH)COOH \longrightarrow Ca[CH_3CH(OH)COO]_2 + CO_2 + H_2O$$

The solubility of calcium lactate is approximately 80g/l in water compared with 15mg/l for $CaCO_3$.

A wide variety of lactic acid bacteria may be used. The choice of species will ultimately be the choice of those of skill in the art and will vary depending upon the conditions encountered in the particular oil well which is to be acidised. Parameters which will influence the choice of species and preferred bacterial species are disclosed below.

In order for the bacteria to produce a quantity of lactic acid in situ sufficient to achieve oil well acidisation, it is desirable to provide a suitable nutrient source in situ. The nutrients may be supplied together with or subsequent to the injection of the bacteria.

In an additional embodiment of the invention, there is provided the use of a lactic acid bacterium in enhancing oil recovery from an oil reserve found in a carbonate rock

formation.

In a still further embodiment of the invention, there is provided the use of a lactic acid bacterium in removing carbonate or iron scale, such as iron oxides or hydroxides in reservoirs and oilfield equipment.

The lactic acid bacteria may be used to remove calcium carbonate scale from sites in oil recovery operations in which scale build up impedes the flow of oil. Such sites include pipework, pumps and tubulars, as well as sandstone reservoirs.

The present invention has the following particular advantages over the prior art:

1) Bacteria can be pumped deep into the formation surrounding the wellbore prior to commencing acid production and calcium carbonate dissolution. They can therefore improve permeability in carbonate reservoirs in a much wider zone around the wellbore and along fractures than hydrochloric acid, which is spent rapidly very close to the wellbore. The use of bacteria in conjunction with horizontal or radial drilling technology improves the access of the bacteria to the oil bearing zone of the reservoir and enhances the treatment benefit.

2) The feedstock for acid production and the bacteria are non corrosive and harmless. This reduces transportation and handling costs, improves the

safety of operatives and eliminates corrosion of equipment at the surface and in the downhole pumps and tubulars.

3) The feedstock is generally of lower cost than hydrochloric acid particularly if the acid contains corrosion inhibitors.

Once a suitable oil field has been identified it is generally necessary to select a suitable microbe(s) that can produce acid under reservoir conditions. The following parameters are relevant in determining the suitability of a particular microorganism:

1) Temperature tolerance-: The temperature of a reservoir is a function of its depth and can be in excess of 100°C. Bacteria can be isolated wherever there is liquid water though the variation of species drops off sharply as temperatures rise above 60°C. Many onshore reservoirs and some offshore reservoirs in carbonate formations are fairly shallow with temperatures falling within the 30- 60°C range. In this temperature range a wide selection of bacterial species can be isolated.

2) Pressure tolerance -: Pressure is also a function of depth; pressures in offshore reservoirs in, for example, the North Sea may exceed 500 atmospheres, whereas shallower on-shore fields are likely to be in the range 50-150 atm. Bacteria can grow at pressures of up to 100 atm, but numbers of species decrease as pressure

increases. The relatively low pressures (<200 atm) found within reservoirs at temperatures below 60°C are unlikely to present a major problem to the lactic acid bacteria. If bacteria are to be injected at rates above the fracture pressure, they must withstand injection pressures which will exceed reservoir pressure.

3) Salt tolerance -: The ability to withstand high salt levels is important as reservoir brines can often be near saturated solutions. Bacterial cultures will be injected in fresh water, but the microbes will need to withstand the effects of salts diffusing into that freshwater.

4) Size -: The ability of the bacteria to enter pores in the rock is vital and where the permeability is very low (<5 mD) the size of the bacteria becomes a limiting factor as the pore throats can be as small as 1μm. Bacteria vary widely in size from <1μm to >20μm in length, depending on species and culture conditions. The lactic acid bacteria range in size from cocci 0.6μm in diameter to rods 9μm long.

5) Resistance to oil toxicity -: Bacteria must be tolerant of oil although they remain in the aqueous phase within the reservoir.

6) Efficiency of acid production -: The ability of bacteria to convert substrate into acid efficiently is fundamental to the process performance. Conversions of

up to 99% of sugar to lactic acid are achieved by some of the lactic acid bacteria.

7) Ability to grow on low cost carbon and nitrogen sources-: The cost of nutrient and microbes for injection should not exceed equivalent hydrochloric acid costs.

The case studies which follow provide examples of this selection process which matches the characteristics of bacteria with those of the reservoir. The selection of a suitable microbe will depend upon obtaining optimum acid production under the target reservoir conditions.

For example, we have found that some species of lactic acid producing bacteria are capable of growth at temperatures up to 54°C; these bacteria were also readily tolerant of pressures of 200 atm indicating that pressure is unlikely to be a limiting factor in determining the microbe to be used.

Some lactic acid bacteria are more tolerant of salt than others. Salt tolerance can be increased by progressive adaptation of the bacteria to increasing salt concentrations up to 11% NaCl.

The efficiency of acid production will usually be the key parameter in selecting a suitable strain of bacterium. However, in situations where permeability of the oil field is very low, it will also be necessary that the microbe is small enough to penetrate the reservoir rock. Under these circumstances, some reduction of the efficiency of acid production may be necessary.

One method for screening lactic acid bacteria to

select a strain compatible with the reservoir conditions for stimulating oil production from an oil well comprises:

(i) selecting candidate strains of lactic acid bacteria with sufficient resistance to oil toxicity such that they are suitable for oil recovery operations;

(ii) determining the temperature, pressure, permeability and salinity of the reservoir;

(iii) determining the temperature, pressure and salinity tolerance of the candidate strains of lactic acid bacteria and selecting strains compatible with the temperature, pressure, permeability and salt conditions measured in (ii) above;

(iv) screening the strains identified in (iii) above for efficiency of lactic acid production and selecting the strain which has an appropriate, preferably the highest, efficiency; and

(v) optionally, if desired, improving the tolerance of the selected lactic acid bacteria to reservoir conditions, such as high salinity, by adaptation.

Adaptation of a selected strain may be performed by conventional techniques known to those of skill in the art. Generally, this involves growing the selected strain under increasing levels of the parameter to which the strain is being adapted, eg salt concentrations.

The optimal strain of bacteria will preferably be selected in step (iv) above in conjunction with a suitable bulk carbon source. Typical carbon sources include sugars, such as glucose, sucrose, dextrose and fructose. A preferred source of carbon is molasses. Preferably, the molasses is from South

Africa or Central America [United Molasses] or an equivalent product with a similar content of fermentable sugars, ie. about 45-75% glucose and fructose. From 1 to 5% for example 2% v/v, is a suitable concentration of molasses in a nutrient medium as a carbon source. Optionally, invertase treatment of the molasses may be performed to increase the availability of reducing sugars. Invertase converts sucrose into glucose and fructose. A concentration of about 0.1% w/v invertase is suitable for this purpose.

In addition to a source of carbon, a source of nitrogen is required. Typical sources of nitrogen include cheese whey and skimmed milk. Cheese whey may be acidic or neutralised. It may be in dried or liquid form. Dried, acidic cheese whey is preferred. Desirably, the cheese whey will have a protein content of from 10 to 15% by weight. A nutrient medium will preferably contain from 1 to 10% w/v cheese whey when the whey is added in solid form. If liquid cheese whey is used, a greater quantity will usually be required since liquid whey has a lower protein content, typically 1%. Cheese whey is a by-product of cheese making, and available from manufacturers in the dairy industry. One suitable source is "Kraffen" (Trade Mark) acidic whey powder from St. Ivel Limited, U.K. Skimmed milk may be in dried or liquid form. It may be spray dried or instantised and will preferably contain 34-37% protein. A suitable source of skimmed milk is that obtained from St. Ivel Ltd.

Alternative sources of nitrogen are yeast extract

and peptone. These components are widely used for microbial culture and are available from a variety of suppliers, eg. Oxoid or Lab M. Either or both of the yeast extract and peptone components may be used in a nutrient medium, for example at a concentration of 0.1 to 20% w/v, preferably from 0.5 to 2% w/v. Other nitrogen sources include ammonium salts, eg ammonium chloride, ammonium phosphate, ammonium sulphate and ammonium nitrate, corn steep liquor, distillers grains and solubles, and soybean flour.

In addition to a carbon and nitrogen source, microbial growth is dependent upon trace amounts of minerals and vitamins. Sodium, potassium, magnesium and manganese salts may be added to a growth medium. We have found that the addition of these metals salts is usually not essential. It is believed that this is because commonly used carbon and nitrogen sources (eg molasses and whey) contain sufficient quantities of such salts. The sulphate and chloride salts of the above metals may be used. Chloride salts are preferred since this will reduce the sulphur content of the medium. This is desirable since this reduces the potential for growth of sulphate reducing bacteria, which may be present in oil wells. Optionally, additional manganese, in the form of manganese chloride or manganese sulphate may be added. Typically, 0.1g/l of a manganese salt may be used. We have found that this may increase acid production in some cases.

Reference may be made to Bergey's Manual of Systematic Bacteriology to determine the vitamin requirements

of the microbe being used. For example, Lactobacillus and Pediococcus species are believed to require pantothenic acid, niacin, riboflavin, folic acid, vitamin B12 and thiamin. These vitamins may be added in trace amounts although we have found that many carbon and nitrogen sources of the type mentioned above contain sufficient trace amounts of these essential requirements.

We have found that two preferred nutrient source for microbial growth comprise:

1)	high test molasses	2% w/v
	Yeast extract (LabM)	1% w/v
	$MnCl_2 . 4H_2O$	0.01% w/v

or

2)	high test molasses	2% w/v
	Kraffen (TM) cheese powder	2% w/v

The case studies which follow show typical screening and selection procedures for suitable bacterial species to use in oil recovery operations. Reference may be made to these case studies in determining further suitable strains for oil fields with different characteristics.

Once a suitable microbe has been identified the treatment can begin. Depending on the character of the rock around the wellbore or the expected length of the fractures the amount of acid needed can be calculated. This will determine

the inoculum size and the amount of nutrient to be employed.

Typically the inoculum is grown up and injected down the production well and a period of time is allowed to elapse to allow penetration of the rock by the bacteria. The injection of bacteria and nutrients may be 'pulsed' by changing the injection rate at intervals in order to enhance the penetration of bacteria through rock pores. The nutrient injection then follows allowing the reaction to start. Alternatively the bacteria may be injected together with the nutrients and time may be allowed at the surface for the fermentation to commence. Usually the process will take between 12 and 168 hours. The well may be shut for all or part of this period to allow the reaction to proceed. Once the process is complete production can begin. When pumping is commenced, injected water containing bacterial fermentation products will be back produced at the surface before oil production starts.

Case studies

Examples of screening programmes to identify candidate bacteria to dissolve calcium carbonate and iron scale in two target reservoirs, one in the US and one in the UK, are presented below.

Parameter	US	UK
Temperature	30°C	50°C
Salinity	3%	7%
Permeability	500 mD	<1 mD
Pressure	600 psi	1500 psi
Type of rock/ scale for dissolution	Calcium Carbonate scale/Iron scale	Calcium carbonate rock
Type of treatment	Matrix acidising	Fracture acidising
Injection pressure	-	3000 psi

It can be seen that there are some significant differences between the two fields and that the organisms selected have to have different characteristics. Due to the low permeability of the UK reservoir, a fracture acidising treatment is likely to be more effective than a matrix treatment. Case study 1 examines the screening procedure for the US reservoir and case study 2 examine the screening procedure for the UK reservoir.

CASE STUDY 1

Choice of Microorganisms

The microbes used in the screen are listed in Table 1. They all produce lactic acid as the major end product of fermentation, with the exception of Clostridium acetobutylicum, which was included for comparison.

Growth of the Bacteria

All the isolates were grown in MRS broth (Oxoid), the composition of which is in the appendix. Apart from Clostridium acetobutylicum all the bacteria were aerotolerant and grown in static culture in universal bottles. Medium was dispensed in 20ml volumes into universal bottles and autoclaved (121°C, 15psi) before being inoculated with a 1% v/v overnight culture. Growth took place in incubators at various temperatures. C. acetobutylicum was grown in bottles sparged with oxygen-free nitrogen; the volume of medium used was 20ml and these cultures also were incubated statically at differing temperatures. To grow the organisms on molasses a modified version of the sucrose medium of Boatwright and Kirsop (appendix) was used.

Measurement of Growth and Acid Production

Growth was measured spectrophotometrically at 560nm

against a sterile MRS medium blank. Acid production was determined after 2-4 days by titrating 10ml of culture medium against 0.1M NaOH. A pH indicator, bromocresol purple (1ml, 0.1% w/v), was used to indicate pH change during titration (yellow to purple at approximately pH 6.8). All the acid production experiments were carried out at 30°C.

Measurement of Calcium Carbonate Dissolution

To test the ability of the cultures to dissolve calcium carbonate, 0.25g of calcium carbonate was added to 50 ml of medium (either molasses or MRS). Dissolution of calcium carbonate was measured by the appearance of soluble calcium in the medium which was assayed in the following way. Alkaline borate buffer (1ml, 0.25M, pH 10.5) was added to 1ml of supernatant and 1ml of o-cresophthalein solution [prepared by dissolving 0.1g o-cresopththalein complexone, 2.5g 8-hydroxyquinoline and 21ml concentrated hydrochloric acid in 11 distilled water]. The purple colour formed was measured spectrophotometrically at 535nm. A standard curve (0-60 μg Ca/ml) was prepared using a solution of $CaCl_2.2H_2O$ (0.275g/l $CaCl_2.2H_2O$ - 100μg Ca/ml).

Measurement of Dissolution of Iron Scale

Dissolution of iron scale (geothite and magnetite was measured by determining the concentration of soluble iron in the culture supernatant (centrifugation: 10,000 rpm, 10 min,

4°C) using the following assay procedure. To 1ml of supernatant was added the following: 0.2ml citric acid (200g/1), 0.001ml concentrated thioglycollic acid, 0.4ml concentrated ammonia solution and 2ml distilled water. After vigorous shaking the absorbance was read spectrophotometrically at 535nm. A standard solution was prepared by dissolving 0.702g $FeSO_4$ $(NH_4)2SO_4.6H_2O$ (ferrous ammonium sulphate) in 300ml water containing 2ml concentrated sulphuric acid. This was diluted to 1l (100μg Fe/ml) and used for the preparation of a standard curve (0-80μg/ml).

Results of Screen

Table 1 shows that all of the bacteria tested were able to grow under the U.S. reservoir conditions and most grew in 3% sodium chloride. The permeability of the US reservoir is sufficiently high that the size of the Lactobacillus rods is not a factor limiting their penetration through the rock. Consequently, the initial choice of organism depended on acid production and scale dissolution rather than simply on the ability to grow under these conditions. In terms of acid yield from dextrose and molasses Lactobacillus plantarum LM1, Pediococcus acidilactici and Lactobacillus sp. all produced a significantly greater quantity of acid than the other microbes tested. All the bacteria tested were effective at dissolving 5g/l calcium carbonate scale and Lactobacillus fermentum and Pediococcus acidilactici were the most effective at dissolving

iron scale. As acid production from a cheap carbon source such as molasses is key to the success of this invention, this criterion had the most weight in the selection. After the initial screen Lactobacillus plantarum was identified as the most suitable organism. Due to its acid yield and ability to dissolve iron scale Pediococcus acidilactici would also be a candidate for the US reservoir.

CASE STUDY 2

To select a microbe for the stimulation of the UK reservoir two properties were paramount: (1) small size (because of the low permeability), and (2) growth at 50°C. A range of microorganisms that are reported to produce acid at 50°C were tested and the results are shown in Table 2. The composition of the media used to grow the microbes is shown in the appendix.

Only four of the organisms tested grew at 50°C and of these the three lactic acid bacteria produced much greater quantities of acid than Clostridium thermosaccharolyticum. Pediococcus acidilactici produced the greatest amount of acid and was the only microbe able to grow in 7% NaCl. It was also able to withstand the injection pressure of 3000 psi necessary to fracture the reservoir. It has a much smaller size than the Lactobacillus species tested and is by far the most suitable bacterium for the stimulation of the UK reservoir.

APPENDIX

Media Compositions

MRS

	g/l
Dextrose	20
Peptone	10
"Lab-Lemco" powder	8
Yeast extract	4
K_2HPO_4	2
Sodium acetate.$3H_2O$	5
Tri-ammonium citrate	2
$MgSO_4.7H_2O$	2
$MnSO_4.4H_2O$	0.05
"Tween 80"	1 ml

The pH of MRS is about 6.2. Additions such as NaCl were added before autoclaving.

Boatwright and Kirsop medium

Molasses	20
Special Peptone	10
Yeast Extract	5
Sodium chloride	5
$MgSO_4.7H_2O$	0.5
$MnCl_2.4H_2O$	0.5
"Tween 80"	1 ml

Clostridium tertium

Medium = Cooked meat Glucose

Cooked meat medium with the supernatant removed and replaced with an equal volume of glucose broth.

Glucose broth = Oxoid nutrient broth + glucose

Lab - lemco beef extract	1.1 g
Yeast extract	2.0 g
Peptone	5.0 g
Glucose	1% w/v
Distilled water	1 l

Clostridium thermoaceticum

$MgSO_4.7H2O$	0.1 g
$(NH_4)2(SO_4)_2$	0.5 g
$Fe(NH_4)_2(SO_4)_2$	0.07 g
$Na_2MoO_4.2H_2O$	2.4 mg
Na_2SeO_3	0.15 mg
Tryptone	5.0 g
Yeast Extract	5.0 g
Resazurin	1.0 mg
K_2HPO_4	7.0 g
KH_2PO_4	4.5 g
Glucose	18.0 g
$NaHCO_3$	10.0 g
Cysteine hydrochloride	0.3 g
Na_2S9H_2O	0.3 g
Distilled water	1 l

Clostridium thermoautotrophicum

Acetobacterium medium:-

NH_4Cl	1.0 g
KH_2PO_4	0.33 g
K_2HPO_4	0.46 g
$MgSO_4 \times 7H_2O$	0.1 g
Trace element soln*	20.0 ml
Vitamin solution *	20.0 ml
Yeast Extract	2.0 g
Fructose	10.0 g
Resazurin	1.0 mg
$NaHCO_3$	1.0 g
Cysteine Hydrochloride	0.5 g
$Na_2S \times 9 H_2O$	0.5 g
Distilled water	1 l

* Trace element solution is, per 1l distilled water: $MgSO_4.7H_2O$, 3.0g; $MnSO_4.2H_2O$, 0.5g; NaCl, 1.0g; $FeSO_4.7H_2O$, 0.1g; $CoSO_4.7H_2O$, 0.18g; $CaCl_2.2H_2O$, 0.1g; $ZnSO_4.7H_2O$, 0.18g; $CuSO_4.5H_2O$, 0.01g; $KAl(SO_4)_2.12H_2O$, 0.02 g; H_3BO_3, 0.01g; $Na_2MoO_4.2H_2O$,0.01g; $NiCl_2.6H_2O$, 0.025 g; $Na_2SeO3.5H_2O$, 0.3g.

Vitamin solution is, per 1l distilled water: Biotin 2mg; Folic acid 2mg, pyridoxine-HCl 10mg; Thiamine-HCl 5mg; Riboflavin 5mg; Nictoinic Acid 5 mg; DL-Ca Pantothinate 5mg; Vitamin B_{12} 0.1mg; p-Amino Benzoic Acid 5mg; Thioctic acid 5mg.

Clostridium thermosaccharolyticum

Fluid thioglycollate medium:

Trypticase	15.0 g
L-cystine	0.5 g
Glucose	5.0 g
Yeast extract	5.0 g
NaCl	2.5 g
Sodium thioglycollate	0.5 g
Resazurin	1.0 mg
Distilled water	1 l

pH = 7.1

Bacillus laevolacticus

Bacillus Racemilacticus Medium

Glucose	5.0 g
Peptone	5.0 g
Yeast extract	5.0 g
$CaCO_3$	5.0 g
Agar	15.0 g
Distilled H_2O	1 l

Sporolactobacillus inulinus

Peotone	10.0 g
Beef extract	10.0 g
Yeast extract	5.0 g
Glucose	20.0g
Tween 80	1.0 ml
K_2HPO_4	2.0 g
Sodium acetate	5.0 g
Triammonoim citrate	2.0 g
$MgSO_4.7H_2O$	0.2 g
$MnSO_4.4H_2O$	0.2 g
$CaCO_2$	30.00 g
Distilled water	1 l

Streptococcus salivarus subspecies thermophilus

Buffered Glucose Broth Media

Oxoid cml broth powder	13.00 g
Glucose	1.00 g
H_2HPO_4	3.68 g
KH_2PO_4	1.32 g
Distilled water	1 l

TABLE 1

Microorganisms	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Lactobacillus fermentum	NCIMB 2797	+	+	rod 1.5-8	0.15	0.72		5*	22. 5
Lactobacillus delbruekii	NCIMB 11778	+	-	rod 2-9	0.97			5	
Lactobacillus acidophilus	NCIMB 1723	+		rod 1.5-6	0.86	0.45		ND	16.0
Lactobacillus plantarum	NCIMB 1406	+	+	rod 3-8	0.54	0.41		5	3.4
Lactobacillus plantarum N	Tate and Lyle	+	+	rod 3-8	1.17	0.99		5	
Lactobacillus plantarum	Tate and Lyle	+	+	rod 3-8	1.28	1.26		5	
Pediococcus acidilactici	NCIMB 12174	+	+	coccus 0.6-1	1.11	1.13	+	5	20.1
Lactobacillus sp	Archaeus Soil Isolate	+	+	rod 1.5-8	1.18	1.35		5	
Clostridium acetobutylicum	NCIMB 8052	+		rod 1.6-6.4	0.54	0		ND	4.4

1 = Source
2 = Growth and acid production at 30°C
3 = Growth and acid production in 3% NaCl
4 = Size (μm)
5 = Acid production from 2% dextrose
6 = Acid production from 2% molasses
7 = Growth and acid prouction at 600 psi
8 = Calcium carboante scale dissolution after 1 day (g/l) (* at least 5g/l since this was the quantity added)
9 = Iron scale dissolution after 28 days (mg/l)

TABLE 2

Microorganisms	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Lactobacillus delbruekiu	NCIMB 8730	+	-	rod 2-9	1.14		
Lactobacillus plantarum (LM1)	Tate and Lyle	+	-	rod 3-8	1.04		
Pediococcus acidilactici	NCIMB 6990	+	+	coccus 0.6-1	1.17	+	17.5
Sporolactobacillus inulinus	NCIMB 9743	-		rod 3-5			
Streptococcus salivarus	NCIMB 8510	-		coccus 0.8-1			
Clostridium tertium	NCIMB 10697	-		rod 1.5-10.2			
C. thermosaccharolyticum	NCIMB 9355	+	-	rod 2.4-16	0.29		
C. thermoautotrophicum	DSM 1974	-		rod 3-6			
C. thermoaceticum	DSM 2910	-					
Bacillus laevolacticus	NCIMB 10269	-		rod 0.4-1			

1 = Source
2 = Growth and acid production at 50°C
3 = Growth and acid production in 7% NaCl
4 = Size (μm)
5 = Acid production at 50°C
6 = Growth and acid prouction at 3000 psi
7 = Calcium Carboante Dissolved from Reservoir Rock (g/l)

CLAIMS

1. A method of stimulating oil recovery from an oil reservoir which comprises:

(i) injecting an inoculum of a lactic acid bacterium compatible with the reservoir conditions into a well bore drilled into the reservoir;

(ii) injecting a source of nutrients for the bacterium into the reservoir;

(iii) allowing the bacterium to ferment thereby producing lactic acid; and

(iv) recovering oil from the reservoir.

2. A method according to claim 1 wherein the inoculum and/or nutrients are injected at a rate above the reservoir fracture pressure.

3. A method according to claim 1 or 2 wherein the oil reservoir is in a carbonate rock structure.

4. A method according to any one of claims 1 or 3 wherein the bacterium is of the genus Lactobacillus, Pediococcus, Streptococcus, Sporolactobacillus, Aerococcus, Bacillus or Leuconostoc.

5. A method according to claim 4 wherein the bacterium is Lactobacillus plantarum or Pediococcus acidilactici.

6. A method according to any of the preceding claims wherein the nutrient source comprises molasses.

7. Use of a lactic acid bacterium in stimulating oil recovery from an oil reserve found in a carbonate rock formation.

8. Use of a lactic acid bacterium in stimulating oil recovery by dissolving carbonate cementation in sandstone reservoirs.

9. Use of a lactic acid bacterium to stimulate oil recovery in reservoirs which have been drilled horizontally or radially.

10. Use of a lactic acid bacterium in removing carbonate or iron scale in oil reservoirs and oilfield equipment.

11. Use of a bacterium according to any one of claims 7 to 10 wherein the bacterium is of the genus Lactobacillus or Pediococcus.

12. Use of a bacterium according to claim 11 wherein the bacterium is Lactobacillus plantarum or Pediococcus acidilacti.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/00378

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 E21B43/25; E21B37/06; E21B43/26; C12S1/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	E21B ; C12P ; C12R

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	NTIS TECH NOTES. no. 9, September 1984, SPRINGFIELD, VA US page 626; TECNICAL INFORMATION CENTER: 'bacteria aid in oil production and utilisation' see the whole document	1-12
Y	US,A,2 852 077 (T.G.COCKS) 16 September 1958 see column 1, line 68 - column 2, line 61; claims 1,3	1-12
Y	US,A,4 749 652 (P.D.ROBISON) 7 June 1988 see claims 1,3	1-12
Y	US,A,4 446 919 (D.O.HITZMAN) 8 May 1984 see claims 1,2	4,5

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27 MAY 1992	04. 06. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ROTSAERT L.D.C.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9200378 SA 57296

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 27/05/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-2852077		None	
US-A-4749652	07-06-88	None	
US-A-4446919	08-05-84	None	